

Genetic analysis of Mendelian mutations in a large UK population-based Parkinson's disease study.

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Abbreviations: UK = United Kingdom; AAO = age at onset; CI = confidence interval; MLPA = Multiplex Ligation-dependent Probe Amplification; MDS-UPDRS = Movement Disorder

29 Society Unified Parkinson's Disease Rating Scale; MoCA = Montreal Cognitive Assessment;
30 LEDD = levodopa equivalent daily dose; MAF = minor allele frequency; SNP = Single
31 Nucleotide Polymorphism; GATK = Genome Analysis Toolkit; PIGD = postural instability gait
32 difficulty; LADS = Leeds Anxiety and Depression Scale; ESS = Epworth Sleep Scale; RBDSQ =
33 Rapid eye movement Sleep Behaviour Disorder Screening Questionnaire; SCOPA = Scales for
34 Outcomes in Parkinson's disease; SD = Standard Deviation.

Abstract

Our objective was to define the prevalence and clinical features of genetic Parkinson's disease in a large UK population-based cohort, the largest multicentre prospective clinico-genetic incident study in the world. We collected demographic data, Movement Disorder Society Parkinson's Disease Rating Scale scores, and Montreal Cognitive Assessment scores. We analysed mutations in *PRKN* (parkin), *PINK1*, *LRRK2* and *SNCA* in relation to age at symptom onset, family history and clinical features.

2,262 participants were recruited to the Tracking Parkinson's study. 424 had young-onset Parkinson's disease (age at onset ≤ 50) and 1,799 had late onset Parkinson's disease. 2005 patients were genotyped with a range of methods. 302 young-onset patients were fully genotyped with Multiplex Ligation-dependent Probe Amplification and either Sanger and/or exome sequencing. 1701 late-onset patients were genotyped with the *LRRK2* 'Kompetitive' allele-specific polymerase chain reaction assay and/or exome sequencing. We identified 29 (1.4%) patients carrying pathogenic mutations. 18 patients carried the G2019S or R1441C mutations in *LRRK2*, and one patient carried a heterozygous duplication in *SNCA*. In *PRKN*, we identified patients carrying deletions of exons 1, 4 and 5, P113Xfs, R275W, G430D and R33X. In *PINK1*, 2 patients carried deletions in exon 1 and 5, and the W90Xfs point mutation.

Eighteen percent of patients with age at onset ≤ 30 and 7.4% of patients from large dominant families carried pathogenic Mendelian gene mutations. Of all young-onset patients, 10 (3.3%) carried bi-allelic mutations in *PRKN* or *PINK1*. Across the whole cohort, 18 patients (0.9%) carried pathogenic *LRRK2* mutations and one (0.05%) carried a *SNCA* duplication. There is a significant burden of *LRRK2* G2019S in patients with both apparently sporadic and familial disease. In young-onset patients, dominant and recessive mutations were equally common. There were no differences in clinical features between *LRRK2* carriers and non-carriers. However, we did find that *PRKN* and *PINK1* mutation carriers have distinctive clinical features compared to early onset non-carriers, with more postural symptoms at diagnosis and less cognitive impairment, after adjusting for age and disease duration. This supports the idea that there is a distinct clinical profile of *PRKN* and *PINK1*-related Parkinson's disease.

63 We estimate that there are approaching 1000 patients with a known genetic aetiology in the UK
64 Parkinson's disease population. A small but significant number of patients carry causal variants
65 in *LRRK2*, *SNCA*, *PRKN* and *PINK1* that could potentially be targeted by new therapies, such as
66 *LRRK2* inhibitors.

67

INTRODUCTION

Parkinson's disease is a progressive neurological condition which affects 140/100,000 people within the UK (Wickremaratchi *et al.*, 2009a). It is caused by genetic mutations in *LRRK2*, *SNCA*, *PRKN* (Parkin or *PARK2*), and *PINK1* in up to 10% of patients (Lesage and Brice, 2012; Puschmann, 2013; Lubbe and Morris, 2014). These genetic factors also influence clinical features of the disease, such as age at onset (AAO) (Cilia *et al.*, n.d.; Clark *et al.*, 2007; Golub *et al.*, 2009; Lesage and Brice, 2012; Klebe *et al.*, 2013), motor features, presenting symptoms, disease progression (Davis *et al.*, 2016) and cognition (Alcalay *et al.*, 2012; Mata *et al.*, 2015; Crosiers *et al.*, 2016).

Many previous studies have focussed on highly selected cohorts recruited from specialist clinics. This is likely to lead to bias both in estimates of frequency and clinical characteristics associated with specific genetic mutations.

In order to overcome these issues, we designed the *Tracking Parkinson's* study, a large-scale population-based prospective cohort study of recently diagnosed and early onset Parkinson's disease patients in the UK. It is the largest single cohort study of genetic mutations in Parkinson's disease and is relatively unbiased. Analysis of this cohort is important to: i.) develop more accurate estimates of genetic risk and the likelihood of a known genetic cause overall and in specific patient sub-groups; ii.) estimate the likelihood of further high risk genes that have not yet been identified and iii.) understand the contribution of Mendelian gene variation to the phenotype of Parkinson's disease.

Several studies have examined the frequency of gene mutations in early onset Parkinson's disease patients (Alcalay *et al.*, 2010a; Kilariski *et al.*, 2012). However, some mutations, such as *LRRK2*, are also present at a significant rate in non-familial late onset Parkinson's disease patients (Clark *et al.*, 2006a). Previous studies have also sometimes used single techniques such as partial Sanger sequencing, which are not able to detect copy number variation common in *PRKN* and less common point mutations. In our analysis, mutations were comprehensively identified using a range of different genetic screening methods, including whole-exome sequencing, Multiplex Ligation-dependent Probe Amplification (MLPA) and Sanger sequencing.

The aim of this study is to describe the frequency of pathogenic Mendelian gene variants in the general Parkinson's disease population and in specific disease sub-groups. In addition, we sought to understand the relationship between Mendelian mutations and clinical phenotype at presentation.

METHODS

Patients were recruited to the *Tracking Parkinson's* study from sites across the UK. Patients were required to have a clinical diagnosis of Parkinson's disease fulfilling Queen Square Brain Bank criteria (Hughes *et al.*, 2001). This project was funded by Parkinson's UK and supported by the National Institute for Health Research.

Patients with disease duration of less than 3.5 years at time of diagnosis were recruited as 'recent onset' participants. Patients with disease duration of greater than 3.5 years at time of diagnosis and AAO \leq 50 years were recruited as 'established young onset' participants. Patients were recruited regardless of ethnicity, including Jewish ethnicity. Full eligibility criteria, exclusion criteria and methods of recruitment have been described previously (Malek *et al.*, 2015b). Importantly, unlike most studies of this type, patients were recruited irrespective of any prior information on genetic status.

Participants' motor features and non-motor features were assessed using standardised and validated scales, including the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Hoehn and Yahr stage and Montreal Cognitive Assessment (MoCA). Full details are provided in the Supplementary Methods.

Pathogenic mutations in the studied genes were defined according to MDSGene (<http://www.mdsgene.org>) (Lill *et al.*, 2016; Kasten *et al.*, 2018), and the Parkinson Disease Mutation Database (PDmutDB; [http://www.molgen.vib-ua.be/Parkinson's diseaseMutDB/](http://www.molgen.vib-ua.be/Parkinson's%20diseaseMutDB/)). Variants that did not meet pathogenicity criteria according to MDSGene (variants classified as 'benign') were not reported.

Genetic analysis of Parkinson's disease gene mutations

At study entry, blood samples were collected from every participant and DNA was extracted from an ethylene diamine tetraacetic acid sample. We screened for mutations in *PRKN*, *PINK1*

and *GBA* with Sanger sequencing. As *GBA* is considered a risk gene for Parkinson's rather than a pathogenic single gene cause, we reported the results of *GBA* sequencing separately (Malek *et al.*, 2018).

Whole exome sequencing was performed in a subset of young-onset and familial patients (N=489) (see Supplementary Methods). Exome sequencing data was screened for pathogenic variants in *SNCA*, *LRR2K2*, *PRKN*, *PINK1*, *DJ-1* and *VPS35*.

Genotyping in young-onset and late-onset patients

2106 patients with Parkinson's disease were genotyped for the *LRRK2* G2019S mutation using the 'Kompetitive' allele-specific polymerase chain reaction (KASP) assay (LGC Genomic Solutions).

We performed SNP array genotyping for 2116 samples. Samples were genotyped using the Illumina HumanCore Exome array supplemented with custom content, including over 27,000 custom variants that have been previously implicated in neurological, neurodegenerative and psychiatric conditions (Malek *et al.*, 2015b). For imputation, genotypes were aligned to the 1000 Genomes Phase 3 v5 mixed population reference panel (Auton *et al.*, 2015) (build hg19/GRCh37) and imputed using Minimac3 (Das *et al.*, 2016) on the Michigan Imputation Server (see Supplementary Methods).

Genotyping in young-onset patients

Patients with AAO ≤ 50 were screened for point mutations in *PRKN* and *PINK1* using Sanger sequencing (Figure 2). We also performed MLPA to detect and confirm copy number variation in *PRKN*, *PINK1*, *DJ1* and *SNCA*. MLPA was performed with the MRC Holland SALSA MLPA P051 Parkinson kit (version D1), according to the manufacturer's instructions. Of 424 patients, 291 (68.7%) were successfully genotyped for *PRKN* and *PINK1* with both MLPA and Sanger sequencing. Eleven patients were screened for copy number variants using MLPA but were not Sanger sequenced. Exome sequencing was performed in 269 patients.

For our final phenotype-genotype analyses, we included young-onset patients if MLPA had been completed, and either Sanger sequencing or exome sequencing, or both, had been completed.

The combination of these methods was selected in order to detect both copy number variants and

point mutations in *PRKN* and *PINK1*. In total, 302 patients with AAO ≤ 50 were included for final analysis.

Genotyping in late-onset patients

Exome sequencing was performed in 219 late-onset patients with a positive family history of Parkinson's disease and 1 patient with missing AAO and a positive family history.

In late-onset patients with 2 or more additional family members affected by Parkinson's disease, MLPA was performed in 65 of 74 (87.8%) patients.

For the final phenotype-genotype analyses, we included late-onset patients if either *LRRK2* KASP genotyping or exome sequencing had been successfully completed. In total, 1701 late-onset patients were included for final analysis, as well as 2 patients with missing AAO.

In total, 2005 patients with Parkinson's disease were included for final analysis (302 young-onset, 1701 late-onset, 2 missing AAO).

Mutations of uncertain pathogenicity

From the exome sequencing data, we report on the frequency of variants that have been previously reported in Parkinson's disease or parkinsonism but whose pathogenicity is uncertain (see Supplementary Methods and Supplementary Table 4).

This study was not designed to confirm pathogenicity of variants through segregation or comparison of allele frequencies in cases and controls. However, we report allele frequencies in our cohort from exome sequencing alongside allele frequencies in controls obtained from gnomAD (<http://gnomad.broadinstitute.org/>).

Haplotype and relatedness analysis

Unimputed genotype data were used for pairwise identity-by-descent (IBD) analysis. Imputed genotype data were used for haplotype analysis. Individual haplotypes were constructed manually for mutation carriers. The markers used to construct haplotypes are detailed in the Supplementary Materials.

Statistical analyses

Demographic characteristics were compared using t-tests, Fisher's exact tests for proportions, or two-sample proportion tests. Linear regression was used for comparisons of demographic characteristics with covariate adjustment. To assess the association between clinical outcomes and genetic status, we used linear regressions of continuous scores against gene status (mutation positive or mutation negative) adjusting for age at assessment, disease duration at study entry, sex and LEDD. Hoehn and Yahr stage, MoCA subdomain and dystonia comparisons were conducted using ordered logistic regression. Motor subtype was analysed using multinomial logistic regression with the tremor dominant group as the comparator. All p-values were 2-tailed. We applied the Bonferroni correction for multiple testing for the number of independent tests in Table 5 and 7. Statistical analysis was conducted using STATA (version 14, StataCorp, Texas, USA) and R (version 3.5.1).

Prevalence estimates

We estimated the absolute numbers of Parkinson's disease patients with a Mendelian genetic cause in the UK using the following approach. We used age-specific prevalence rates from a previous UK meta-analysis (Wickremaratchi *et al.*, 2009b) and applied the rates to the Office of National Statistics Great Britain mid-2016 population estimates (Office for National Statistics, 2017) to derive an approximate number of all Parkinson's disease patients. The age distribution of the Parkinson's disease population (as a percentage) was used to standardise the rates of genetic Parkinson's disease within our cohort (per 100,000). From this, we derived the new age-standardised rate of genetic Parkinson's disease. We applied this age-standardisation method because our over-sampling of young onset cases has resulted in a non-representative age-distribution of patients. This new rate was then applied to the total Parkinson's disease population to estimate the absolute number of patients with a Mendelian genetic cause in the UK population. It is important to note that as we have derived the rates from our incident cases (excluded established young onset cases), we have assumed that the rates are representative of all prevalent cases. This may not be true if these Mendelian forms of Parkinson's disease are associated with better or worse survival, in which case our estimates will be either an under- or over-estimate of the true numbers. 95% confidence intervals were calculated using the Poisson distribution.

208 *Data availability*

209 The anonymised data from this study are available to researchers, to support other studies. Please
210 apply via the Tracking Parkinson's project coordinator (tracking-parkinsons@glasgow.ac.uk).

211 **RESULTS**

212 Table 1 shows the baseline demographics for participants that met Parkinson's disease diagnostic
213 criteria. Data are presented separately for three groups below, according to inclusion criteria for
214 recruitment. Young onset patients were separated into recent and established patients, as only the
215 recent onset patients represent an incident, largely population-based cohort of Parkinson's
216 disease. For this reason, only recent onset patients were used to estimate the prevalence of
217 genetic forms of Parkinson's disease in the UK.

- 218 1) Recent late onset Parkinson's disease patients (AAO > 50, disease duration \leq 3.5 years at
219 time of diagnosis),
- 220 2) Recent young onset Parkinson's disease patients (AAO \leq 50, disease duration \leq 3.5 years
221 at time of diagnosis)
- 222 3) Established young onset Parkinson's disease patients (AAO \leq 50, disease duration > 3.5
223 years at time of diagnosis).

224 37 patients received a revised alternative diagnosis other than Parkinson's disease or had
225 conflicting dopamine transporter (DaT) scan results and were excluded from further analysis. On
226 rare occasions, *LRRK2* mutations may be present in progressive supranuclear palsy or atypical
227 parkinsonian patients (Sanchez-Contreras *et al.*, 2017; Vilas *et al.*, 2017), however we did not
228 identify any pathogenic mutations in these patients.

229 Table 1. Baseline demographics for all Parkinson's disease patients with known AAO.

	Recent, late onset patients (AAO>50, ≤3.5 years from diagnosis) N=1799	Recent, young onset patients (AAO≤50, ≤3.5 years from diagnosis) N=197	Established young onset patients (AAO≤50, >3.5 years from diagnosis) N=227	Total N=2223
Age at recruitment (years)	69.3 (7.5)	48.8 (6.2)	54.5 (7.7)	66.0 (10.2)
Age at onset (years)	66.4 (7.7)	43.7 (5.6)	41.1 (7.1)	61.8 (12.1)
Disease duration at diagnosis (years)	1.3 (0.9)	1.4 (1.0)	11.4 (6.4)	2.4 (3.8)
Disease duration at entry (years)	2.9 (2.1)	5.2 (6.6)	13.1 (7.4)	4.0 (4.6)
Family history (n, (%))				
No family history	1442 (80.2%)	145 (73.6%)	166 (73.1%)	1753 (78.9%)
1 additional affected family member	267 (14.8%)	41 (20.8%)	47 (20.7%)	355 (16.0%)
2 additional affected family members	59 (3.3%)	8 (4.1%)	8 (3.5%)	75 (3.4%)
3 additional affected family members	11 (0.6%)	2 (1.0%)	4 (1.8%)	17 (0.8%)
4 or more additional affected family members	4 (0.2%)	0 (0.0%)	1 (0.4%)	5 (0.2%)
Consistent with dominant inheritance	305 (17.0%)	49 (24.9%)	57 (25.1%)	411 (18.5%)
Consistent with recessive inheritance	36 (2.0%)	2 (1.0%)	3 (1.3%)	41 (1.8%)
Consanguinity				
Non-consanguineous	1741 (96.8%)	191 (97.0%)	220 (96.9%)	2152 (96.8%)
Consanguineous	16 (0.9%)	2 (1.0%)	2 (0.9%)	20 (0.9%)

Ethnicity				
White	1742 (96.8%)	188 (95.4%)	211 (93.0%)	2141 (96.3%)
Asian or Asian British	16 (0.9%)	3 (1.5%)	8 (3.5%)	27 (1.2%)
Black or Black British	10 (0.6%)	3 (1.5%)	2 (0.9%)	15 (0.7%)
Chinese	0 (0.0%)	0 (0.0%)	2 (0.9%)	2 (0.1%)
Mixed	4 (0.2%)	0 (0.0%)	0 (0.0%)	4 (0.2%)
Other	2 (0.1%)	1 (0.5%)	0 (0.0%)	3 (0.1%)
Sex				
Male	1181 (65.7%)	124 (62.9%)	149 (65.6%)	1454 (65.4%)

230 AAO= age at onset

231 Consistent with dominant inheritance=family members from multiple generations affected

232 Consistent with recessive inheritance=family members only from the same generation affected

233

234 *Summary of genotyping*

235 Supplementary Figures 1 to 5 show the number of patients that were genotyped for each method.

236 The shaded boxes highlight the samples that were included for analysis. There were

237 approximately 100 patients for which DNA was not available for genotyping (this varied

238 between different methods). These patients were excluded from phenotype-genotype analyses.

239 For young-onset patients, we included samples for final analysis if MLPA had been completed,

240 and either Sanger sequencing or exome sequencing or both had been successfully completed. In

241 total, 302 patients with $AAO \leq 50$ were included for final analysis of *PRKN* and *PINK1*.

242 For late-onset patients, we included patients for final analysis if the samples had been genotyped

243 with the *LRRK2* KASP assay for G2019S, and/or exome sequencing. In total, 1701 late-onset

244 patients were included for final analysis, as well as 2 patients with missing AAO.

245 In total, 2005 patients with Parkinson's disease were included for final analysis (302 young-

246 onset, 1701 late-onset, 2 missing AAO).

247

248 *Summary of mutations identified*

249 We identified 14 different pathogenic mutations in *LRRK2*, *SNCA*, *PRKN* and *PINK1* in 29 out
250 of 2005 patients (1.4%, 95% CI 0.9-2.0%) (Tables 2 and 3). This estimate is conservative as not
251 all samples were comprehensively tested, therefore the true mutation rate may be higher.

252 18 patients carried a mutation in *LRRK2*, 1 patient carried a *SNCA* mutation, 8 patients carried
253 biallelic *PRKN* mutations and 2 patients carried biallelic *PINK1* mutations. No patients were
254 found carrying pathogenic mutations in *VPS35* or *DJI*. No patient carried pathogenic mutations
255 in more than one gene. 3 patients carried the *LRRK2* G2019S mutation and additionally one or
256 more mutations in *GBA* (p.E326K and p.P122H). The mean AAO for patients carrying mutations
257 in both *LRRK2* and *GBA* mutations was 43.2 years (SD=5.1), compared to an AAO of 56.5 years
258 (SD=12.9) for *LRRK2* mutation carriers without *GBA* mutations. Pathogenic mutation carriers
259 are shown in Supplementary Table 1 and the list of unique mutations are shown in
260 Supplementary Table 2.

261 We identified 9 patients carrying single heterozygous pathogenic mutations in *PRKN* and *PINK1*
262 (Supplementary Table 3). Previous analysis of this cohort showed no differences between
263 carriers of single heterozygous *PRKN* mutations (including mutations of uncertain pathogenicity)
264 and non-carriers other than in olfaction (Malek *et al.*, 2015a), therefore patients with single
265 heterozygous mutations in recessive genes were analysed as non-carriers.

266 One patient carried 3 pathogenic mutations in *PRKN* (Supplementary Table 1).

267 Mutations were common in patients with very young onset and patients with multiple family
268 members also affected by Parkinson's disease. 18.8% (3/16; 95% CI 6.6 – 43.0%) of Parkinson's
269 disease patients with onset \leq 30 carried pathogenic mutations. In young-onset patients, 18.2%
270 (4/22; 95% CI 7.3 – 38.5%) of patients with 2 or more additional affected family members
271 carried pathogenic mutations. In late-onset patients, 4.2% (3/72; 95% CI 1.4-11.5%) of patients
272 with 2 or more additional affected family members carried pathogenic mutations.

273 Notably, the *LRRK2* G2019S mutation was more common in young onset patients (2.2%, 9/408;
274 95% CI 0.7 – 3.6%) than in later onset patients (0.4%, 7/1701; 95% CI 0.1 – 0.7%), $p=0.001$

275 (Fisher's exact test, OR = 5.5, 95% CI 1.8-17.3). In addition, early onset patients were equally
276 likely to have recessive (2.5%, 10/408) and dominant pathogenic mutations (2.2%, 9/408).

277 Pathogenic mutations were only identified in patients reporting 'White' ethnicity (N=2005
278 genotyped).

279 IBD analysis was conducted based on 25,781 SNPs in linkage equilibrium. This showed that
280 none of the mutation carriers were related to each other (π -hat <0.1 for all, indicating no closer
281 relations than third-degree relatives).

282 Constructed haplotypes and the results of haplotype analysis are shown in the Supplementary
283 Materials (Supplementary Figures 6 to 9).

284

Table 2. Overall frequency of dominant gene mutation carriers for known pathogenic variants in successfully genotyped patients. Percentages and 95% CIs are shown in brackets.

	Young onset N=408	Late onset N=1701	All N=2003
<i>LRRK2</i>	9 (2.2%; 0.8-3.6%)	9 (0.5%; 0.2-0.9%)	18 (0.9%; 0.5-1.3%)
<i>SNCA</i>	0 (0%; 0.0 – 0.9%)	1 (0.06%; 0.01-0.3%)	1 (0.05%; 0.04-0.1%)
All autosomal dominant (<i>LRRK2</i> and <i>SNCA</i>)	9 (2.2%; 0.8-3.6%)	10 (0.6%; 0.2-1.0%)	19 (0.9%; 0.5-1.4%)

Table 3. Overall frequency of biallelic recessive gene mutation carriers for known pathogenic variants in successfully genotyped young onset patients (AAO \leq 50). Percentages and 95% CIs are shown in brackets.

PRKN	Young onset N = 302
Homozygous	0 (0%; 0.0-0.1.3%)
Compound heterozygous	8 (2.6%; 0.8-4.5%)
PINK1	
Homozygous	1 (0.3%; 0.06-1.9%)
Compound heterozygous	1 (0.3%; 0.06-1.9%)
All autosomal recessive (<i>PRKN</i> and <i>PINK1</i> biallelic mutations)	10 (3.3%; 1.3-5.3%)

292 Table 4. Rate of known dominant pathogenic mutations based on clinical presentation.

	<i>LRRK2</i> N=18	<i>SNCA</i> N=1	Rate of all pathogenic dominant mutations
Age at onset			
≤20 years (N=4)	0/4 (0%)	0/4 (0%)	0/4 (0%)
≤30 years (N=18)	0/18 (0%)	0/18 (0%)	0/18 (0%)
≤40 years (N=118)	2/118 (1.7%)	0/118 (0%)	2/118 (1.7%)
≤50 years (N=408)	9/408 (2.2%)	0/408 (0%)	9/408 (2.2%)
≤60 years (N=784)	10/784 (1.3%)	1/784 (0.1%)	11/784 (1.4%)
≤70 years (N=1552)	17/1552 (1.1%)	1/1552 (0.06%)	18/1552 (1.2%)
≤80 years (N=2050)	18/2050 (0.9%)	1/2050 (0.05%)	19/2050 (0.9%)
All (N=2109)	18/2109 (0.9%)	1/2109 (0.05%)	19/2109 (0.9%)
Mean age of onset in years (SD)	54.3 (12.9)		54.1 (12.6)
Family history			
No other family members affected	8/1658 (0.5%)	0/1658 (0%)	8/1658 (0.5%)
1 other family member affected	7/344 (2.0%)	0/344 (0%)	7/344 (2.0%)
2 other family members affected	1/72 (1.4%)	1/72 (1.4%)	2/72 (2.8%)
3 other family members affected	2/17 (11.8%)	0/17 (0%)	2/17 (11.8%)
4 or more family members affected	0/5 (0%)	0/5 (0%)	0/5 (0%)

293

294 *LRRK2*

295 We identified 18 patients carrying heterozygous *LRRK2* mutations, either G2019S (N=16) or
 296 R1441C (N=2). 55.6% (10/18) carriers reported a positive family history of Parkinson's disease.

297

Both *LRRK2* R1441C carriers reported a family history of Parkinson's disease. As we only screened for the R1441C mutation through exome sequencing in familial and/or young-onset patients, our results for R1441C cannot be used to compare familial vs. non-familial patients.

We only included *LRRK2* G2019S mutation carriers for our analysis of family history. G2019S mutations were more common among patients with a positive family history (1.9%, 95% CI 0.5-3.1%) than patients without a family history of Parkinson's disease (0.5%, 95% CI 0.1-0.8%), $p=0.009$ (Fisher's exact test, OR = 3.9, 95% CI 1.3-11.8). However, within the G2019S carriers, 50% had a positive family history and 50% did not have a family history of Parkinson's (50%, 95% CI 25.5-74.5%).

LRRK2 mutation carriers (G2019S and R1441C carriers together) had an earlier mean AAO (54.3 years, 95% CI 47.9-60.7) compared to non-carriers (61.7 years, 95% CI 61.2-62.2; $p=0.01$). AAO for *LRRK2* carriers ranged from 35.2 to 78.7 years. *LRRK2* mutations were more frequent in early onset (2.2%, 95% CI 1.0-4.2%) compared to late onset patients (0.5%, 95% CI 0.2-1.0%), $p=0.003$ (Fisher's exact test, OR = 4.2, 95% CI = 1.5-12.1).

Clinical features of *LRRK2* carriers compared to non-carriers are presented in Table 5 (excluding patients with recessive gene mutations). We did not include the *SNCA* carrier in this analysis given that previous literature suggests that *LRRK2* and *SNCA* mutation carriers have different clinical features (Trinh *et al.*, 2018). We did not find any differences in clinical features between *LRRK2* carriers and non-carriers.

SNCA

SNCA copy number variants were screened with MLPA in 65 patients with familial Parkinson's disease with 2 or more family members affected. One patient (1.5%) carried a heterozygous whole gene duplication was identified, who reported 2 additional family members affected by Parkinson's disease. We were unable to compare the clinical features of *SNCA* carriers to non-carriers given that only one *SNCA* carrier was identified.

Table 5. Comparison of motor features, fluctuations and non-motor features by *LRRK2* mutation status (*LRRK2* carriers vs. non-carriers). Patients carrying biallelic recessive mutations and one patient carrying a *SNCA* mutation were excluded from analyses. Scores in the first 2 columns are means (SD), except for Hoehn and Yahr stage, symptoms present at diagnosis and motor subtype which are shown as N or proportions (%). Increasing scores and increasing beta values for motor and non-motor variables are associated with worse symptoms, with the exception of the MoCA test scores. Increasing scores and increasing beta values for the MoCA test are associated with better cognition.

Variable	Mutation negative N=2082	LRRK2 positive N=18	Beta (95% CI) LRRK2 carriers vs. non-carriers	p-value ^a
Age at entry (years)	66.0 (10.1)	60.1 (10.4)	-5.2 (-9.9, -0.5)	0.030^b
Age at onset (years)	61.8 (11.9)	54.3 (12.9)	-5.2 (-9.9, -0.5)	0.030^b
Disease duration (years)	4.0 (4.4)	5.2 (4.5)	0.7 (-1.3, 2.8)	0.482 ^c
Delay to diagnosis (time from symptom onset to diagnosis) (years)	1.8 (2.9)	1.5 (1.3)	-0.4 (-1.8, 1.0)	0.580 ^c
Motor features				
MDS-UPDRS III total score	23.4 (12.7)	28.6 (15.2)	6.7 (0.1, 13.3)	0.047
Severity score MDS-UPDRS-III/years from symptom onset	10.4 (11.8)	9.4 (7.3)	0.6 (-5.7, 6.8)	0.862 ^d
Upper limb score (max 56)	10.7 (6.3)	12.1 (6.3)	2.1 (-0.9, 5.1)	0.163
Lower limb score (max 32)	5.1 (3.9)	6.8 (5.5)	1.7 (-0.2, 3.6)	0.085
Gait and freezing (max 8)	1.1 (1.1)	1.6 (1.7)	0.4 (-0.1, 0.9)	0.097

Hoehn and Yahr stage			0.3 (-0.7, 1.2)	0.595
0-1.5 (%)	950 (46.0%)	7 (38.9%)		
2 or 2.5 (%)	957 (46.3%)	10 (55.6%)		
3+ (%)	160 (7.7%)	1 (5.6%)		
Symptoms present at diagnosis				
Tremor	1499/2017 (74.3%)	13/18 (72.2%)	0.3 (-0.8, 1.6)	0.586
Rigidity	1410/1925 (73.2%)	13/18 (72.2%)	-0.08 (-1.2, 1.2)	0.891
Bradykinesia	1554/1966 (79.0%)	12/18 (66.7%)	-0.8 (-1.8, 0.3)	0.121
Postural problems	363/1898 (19.1%)	4/18 (22.2%)	0.009 (-1.5, 1.2)	0.989
Other	456/1827 (25.0%)	4/16 (25 %)	0.2 (-1.1, 1.3)	0.731
Motor subtype				
Tremor dominant	835/1892 (44.1%)	7/17 (41.2%)		
Non-tremor dominant (PIGD)	813/1892 (43.0%)	10/17 (58.8%)	-2.8 (-0.5, 1.8)	0.246
Mixed	244/1892 (12.9%)	0/17 (0%)	-8.7 (NA)*	NA*
Motor complications				
MDS-UPDRS-IV total score	1.3 (2.8)	2.8 (3.3)	0.1 (-0.9, 1.2)	0.794
Dyskinesias (MDS-UPDRS IV part 1 and 2 sum - max 8)	0.3 (1.0)	0.4 (0.9)	-0.2 (-0.5, 0.1)	0.259
Fluctuations (MDS-UPDRS IV part 3, 4 and 5 sum - max 12)	0.9 (1.9)	2.1 (2.6)	0.3 (-0.4, 1.1)	0.408
Dystonia (max 4)	0.2 (0.6)	0.3 (0.6)	0.01 (-0.2, 0.3)	0.915
Non-motor features				

Cognition - total MoCA score	25.2 (3.5)	25.4 (3.2)	-0.2 (-1.9, 1.4)	0.761
Visuospatial (max 5)	4.3 (1.1)	4.2 (1.2)	-0.2 (-0.7, 0.3)	0.359
Naming (max 3)	2.9 (0.3)	2.9 (0.3)	-0.05 (-0.2, 0.1)	0.535
Attention (max 6)	5.2 (1.0)	5.3 (0.8)	0.1 (-0.4, 0.6)	0.690
Language (max 3)	2.4 (0.8)	2.4 (0.7)	-0.03 (-0.4, 0.3)	0.865
Abstraction (max 2)	1.6 (0.6)	1.7 (0.7)	0.003 (-0.3, 0.3)	0.983
Recall (max 5)	2.7 (1.6)	2.9 (1.8)	0.05 (-0.7, 0.8)	0.898
Orientation (max 6)	5.8 (0.5)	5.8 (0.5)	-0.03 (-0.2, 0.2)	0.756
LADS Anxiety score (max 18)	4.5 (3.8)	5.8 (3.8)	0.9 (-0.8, 2.6)	0.287
LADS Depression score (max 18)	4.5 (3.3)	5.1 (3.3)	0.3 (-1.2, 1.8)	0.706
Sleep disturbance (ESS score)	7.1 (4.8)	9.7 (6.8)	1.6 (-0.7, 3.8)	0.173
REM Sleep Behaviour Disorder (RBDSQ) scale score	4.8 (3.2)	6.4 (3.5)	1.0 (-0.5, 2.5)	0.191
Autonomic function: SCOPA total score	9.3 (5.8)	10.8 (6.4)	2.6 (-1.1, 6.3)	0.170

SD = standard deviation; CI = confidence interval; MDS-UPDRS = Movement Disorder Society Unified Parkinson's Disease Rating Scale; PIGD = postural instability gait difficulty; MoCA= Montreal Cognitive Assessment; LADS = Leeds Anxiety and Depression Scale; ESS= Epworth Sleep Scale; RBDSQ = Rapid Eye Movement Sleep Behaviour Disorder Screening Questionnaire; SCOPA = Scales for Outcomes in Parkinson's disease.

^a *P* value of clinical features of *LRRK2* carriers together compared to non-carriers, excluding patients with recessive gene mutations and one patient with *SNCA* mutation. Adjusting for age at entry, gender, disease duration at entry/assessment and LEDD total, unless otherwise specified.

336 ^b Adjusting for gender and disease duration at entry

337 ^c Adjusting for gender and age at entry.

338 ^d Adjusting for age, gender and LEDD total.

339 *Insufficient count to fit model

340 *Young-onset patients*

341 We identified 19/302 (6.3%) young-onset patients carrying pathogenic mutations in both
342 dominant and recessive genes. The proportions of mutation carriers by AAO and family history
343 are presented in Table 6. Recessive gene mutation carriers had an earlier mean AAO (32.7 years)
344 compared to non-carriers (41.1 years), $p < 0.001$, excluding dominant mutation carriers.

345 When considering all young-onset mutation carriers (*PRKN*, *PINK1*, *LRRK2* and *SNCA*)
346 mutation carriers, the mean AAO was also younger than non-carriers (37.5 vs. 41.1 years;
347 $p = 0.02$). Mutations were more frequent in patients with a positive family history (11.0%) than in
348 patients with no family history of Parkinson's disease (4.2%), $p = 0.04$ (Fisher's exact test, OR =
349 2.8, 95% CI 1.0-8.1).

350

Table 6. Cumulative rate of pathogenic mutations based on clinical presentation in successfully genotyped early onset Parkinson's disease patients (AAO \leq 50), N=302.

	<i>PINK1</i> (biallelic) N=2	<i>PRKN</i> (biallelic) N=8	All recessive gene mutations N=10
Age at onset			
\leq 20 years (N=4)	0/4 (0%)	2/4 (50%)	2/4 (50%)
\leq 30 years (N=18)	0/16 (0%)	3/16 (18.8%)	3/16 (18.8%)
\leq 40 years (N=118)	1/110 (0.9%)	6/110 (5.5%)	7/110 (6.4%)
\leq 50 years (N=408)	2/302 (0.7%)	8/302 (2.6%)	10/302 (3.3%)
Mean age of onset in years (SD)	42.3 (5.5)	30.3 (11.5)	
Family history			
No other family members affected	1/213 (0.5%)	4/213 (1.9%)	5/213 (2.3%)
1 other family member affected	1/67 (1.5%)	1/67 (1.5%)	2/67 (3.0%)
2 other family members affected	0/15 (0%)	3/15 (20%)	3/15 (20%)
3 other family members affected	0/6 (0%)	0/6 (0%)	0/6 (0%)
4 or more other family members affected	0/1 (0%)	0/1 (0%)	0/1 (0%)

353

354 *PRKN*

355 Of all young-onset patients that were successfully genotyped for *PRKN*, biallelic pathogenic
 356 *PRKN* mutations were present in 2.6% (8/302, 95% CI 0.8-4.4%). No *PRKN* carriers had
 357 homozygous mutations; all mutations were present in compound heterozygous state.

358 *PRKN* mutations were present in 20% (3/15, 95% CI 7.0-45.2%) of early onset patients with 2
 359 additional family members affected by Parkinson's disease. However, there was no significant
 360 difference in the frequency of mutations in early onset patients with a positive family history
 361 (4.2%, 95% CI 0.2-8.4%) and without a family history of Parkinson's disease (1.9%, 95% 0.05-
 362 3.7%), $p > 0.2$ (Fisher's exact test, OR = 2.3, 95% CI 0.4-12.9). Young-onset patients from large

Parkinson's disease families (2 or more additional family members affected) were more likely to carry a *PRKN* mutation (13.6%) than early onset patients with 1 or no additional family members affected (1.6%), $p=0.01$ (Fisher's exact test, OR = 8.5, 95% CI 1.2-47.9).

The clinical features of *PRKN* and *PINK1* mutation carriers compared to early-onset non-carriers are presented in Table 7. *PRKN* carriers had younger AAO than early onset patients with *LRRK2* mutations (42.9 years, 95% CI 39.3-46.6), $p=0.009$. There was no difference in AAO of *PRKN* and *PINK1* carriers, $p>0.2$.

PINK1

Bi-allelic *PINK1* mutations were present in 0.7% (2/302, 95% CI 0.2-2.4%) of all screened young-onset patients. Mutations were present in 1.1% (1/89) of young-onset patients with a positive family history and 0.5% (1/213) of patients with no family history of Parkinson's disease. Mutations were not more frequent with patients with a positive family history, $p=0.50$ (Fisher's exact test, OR = 2.4, 95% CI 0.03-189.7).

PRKN and *PINK1* mutation carriers had earlier age at study entry and earlier AAO than other early-onset non-carriers, adjusting for gender and disease duration (Table 7). They also had longer disease duration than non-carriers, adjusting for age at entry and gender (Table 7).

PRKN and *PINK1* mutation carriers also reported more postural problems at diagnosis than non-carriers and tended to report a higher rate of dyskinesias, after adjusting for age at entry, gender, disease duration and LEDD total, although this did not survive correction for multiple testing. They also tended to have more gait and freezing problems at assessment, after adjusting for age, gender, disease duration and LEDD total ($p=0.021$), although this was not significant after correction for multiple testing.

Finally, *PRKN* and *PINK1* carriers had better cognition than non-carriers as assessed by the MoCA, even after adjusting for age, gender, disease duration and LEDD ($p=0.007$). This appears to be driven by better performance in the attention subdomain ($p=0.004$) though one must be cautious in interpreting the sub-domains as they may be overly simplistic.

390 *Genes of unconfirmed pathogenicity for Parkinson's disease*

391 Patients carrying variants of unconfirmed pathogenicity and risk variants for Parkinson's disease
392 identified from exome sequencing are reported in Supplementary Table 4, including variants in
393 *GIGYF2*, *CHDCHD2*. These variants were detected in cases, as previously described, but also
394 almost all occur in the control population and were not included as pathogenic variants in our
395 analysis.

396 We found comparable mutation/variant frequencies in our cohort compared to controls, with the
397 exception of well-validated risk variants, such as *MAPT* (Martin *et al.*, 2001; Kwok *et al.*, 2004).
398 We did not find any patients carrying previously reported mutations in *EIF4G1*, *DNAJC6*,
399 *FBXO7* and *PLA2G6*. Further case-control studies are needed to determine the role of variants in
400 *SNCAIP*, *UCHL1* and other genes where we found small differences in allele frequencies from
401 control frequencies, however these variants are unlikely to be pathogenic Mendelian mutations.

Table 7. Comparison of motor features, fluctuations and non-motor features of early onset patients by recessive gene status (*PRKN* and *PINK1* carriers vs. non-carriers), excluding patients carrying dominant gene mutations. Scores in the first 4 columns are means (SD), except for Hoehn and Yahr stage, symptoms present at diagnosis and motor subtype which are shown as N or proportions (%). Increasing values and increasing betas for motor and non-motor variables are associated with worse symptoms, with the exception of the MoCA test scores. Increasing values and increasing betas for the MoCA test are associated with better cognition. Cells with only a single case are indicated with brackets (N=1).

Variable	Mutation negative	Mutation positive (bi-allelic)			Beta (95% CI)	p-value ^a
	N=292	Total N=10	<i>PRKN</i> N=8	<i>PINK1</i> N=2	Carriers vs. non-carriers	
Age at entry (years)	51.9 (8.1)	50.9 (11.1)	51.8 (12.2)	47.5 (5.9)	-7.0 (-10.9, -3.1)	0.001^b
Age at onset (years)	41.1 (6.2)	32.7 (11.5)	30.3 (11.5)	42.3 (5.5)	-7.0 (-10.9, -3.1)	0.001^b
Disease duration (years)	10.4 (7.6)	18.2 (14.4)	21.9 (14.4)	5.2 (0.4)	8.9 (5.0, 12.7)	<0.001^c
Delay to diagnosis (years)	2.4 (4.2)	4.5 (4.1)	5.2 (4.4)	2.2 (0.1)	2.2 (-0.6, 5.1)	0.123 ^c
Motor features						
MDS-UPDRS-III total score	26.1 (14.9)	29.0 (24.0)	33.0 (23.6)	5.0 (N=1)	-3.3 (-14.4, 7.8)	0.564
Severity score MDS-UPDRS-III/years from symptom onset	4.1 (6.8)	2.4 (2.9)	2.7 (3.1)	0.9 (N=1)	-2.5 (-7.7, 2.8)	0.356 ^d
Upper limb score (max 56)	11.6 (6.7)	13.9 (8.8)	15.3 (8.7)	8.5 (9.2)	-1.1 (-5.5, 3.3)	0.621
Lower limb score (max 32)	6.2 (4.4)	7.7 (5.6)	8.5 (6.0)	4.5 (3.5)	-0.1 (-3.1, 3.0)	0.973
Gait and freezing (max 8)	1.6 (1.5)	3.2 (1.9)	3.6 (1.7)	1.5 (2.2)	1.1 (0.03, 2.1)	0.043

Hoehn & Yahr stage					1.8 (0.1, 3.6)	0.049
0-1.5 (%)	107 (36.7%)	1 (11.1%)	1 (12.5%)	0 (0%)		
2 or 2.5 (%)	140 (48.1%)	4 (44.4%)	3 (37.5%)	1 (100%)		
3+ (%)	44 (15.1%)	4 (44.4%)	4 (50%)	0 (0%)		
Symptoms present at diagnosis						
Tremor	188/263 (71.5%)	7/10 (70.0%)	6/8 (75.0%)	1/2 (50.0%)	-0.9 (-2.4 0.8)	0.275
Rigidity	204/255 (80%)	8/9 (88.9%)	6/7 (85.7%)	2/2 (100%)	0.7 (-1.2, 3.7)	0.561
Bradykinesia	209/257 (81.3%)	9/10 (90.0%)	7/8 (87.5%)	2/2 (100%)	15.1 (-55.4, NA)	0.986
Postural problems	39/252 (15.5%)	6/9 (66.7%)	6/7 (85.7%)	0/2 (0%)	2.3 (0.7, 4.0)	0.005
Other	54/229 (23.6%)	3/9 (33.3%)	3/7 (42.9%)	0/2 (0%)	0.4 (-1.6, 2.0)	0.684
Motor subtype (%)						
Tremor dominant	79/257 (30.7%)	2/8 (25.0%)	1/6 (16.7%)	1/2 (50%)		
Non-tremor dominant (PIGD)	150/257 (58.4%)	6/8 (75.0%)	5/6 (83.3%)	1/2 (50%)	0.4 (-1.4, 2.3)	0.646
Mixed/ Indeterminate	28/257 (10.9%)	0/8 (0%)	0/6 (0%)	0/2 (0%)	-9.5 (NA, NA)	>0.1

Motor complications						
MDS-UPDRS-IV total score	5.0 (4.9)	6.2 (5.7)	6.1 (6.3)	6.5 (3.5)	2.3 (-0.5, 4.5)	0.105
Dyskinesias (presence and severity; max 8)	1.3 (1.9)	2.3 (2.5)	2.1 (2.8)	3.0 (1.4)	1.2 (0.03, 2.3)	0.04
Fluctuations (max 12)	3.0 (2.9)	3.3 (4.0)	3.4 (4.3)	3.0 (4.2)	0.9 (-0.8, 2.6)	0.309
Dystonia (max 4)	0.7 (1.1)	0.6 (1.3)	0.6 (1.4)	0.5 (0.7)	0.1 (-0.7, 0.8)	0.891
Non-motor features						
Cognition - total MoCA score (max 30)	25.6 (3.6)	27.6 (2.2)	27.4 (2.3)	29.0 (N=1)	3.0 (0.8, 5.2)	0.007
Visuospatial (max 5)	4.4 (1.1)	4.3 (0.5)	4.4 (0.5)	4.0 (N=1)	0.07 (-0.6, 0.8)	0.847
Naming (max 3)	2.9 (0.3)	2.9 (0.3)	2.9 (0.4)	3.0 (0.0)	0.08 (-1.2, 0.3)	0.441
Attention (max 6)	5.1 (1.0)	5.6 (0.5)	5.5 (0.5)	6.0 (0.0)	0.9 (0.3, 1.6)	0.004
Language (max 3)	2.5 (0.7)	2.3 (0.8)	2.4 (0.7)	2.0 (1.4)	-0.07 (-0.5, 0.4)	0.767
Abstraction (max 2)	1.7 (0.6)	1.6 (0.7)	1.6 (0.7)	1.5 (0.7)	0.09 (-0.4, 0.5)	0.704
Recall (max 5)	3.1 (1.6)	4.2 (1.3)	4.3 (1.4)	4.0 (1.4)	0.9 (-0.2, 2.0)	0.116
Orientation (max 6)	5.7 (0.7)	6.0 (0.0)	6.0 (0.0)	6.0 (0.0)	0.3 (-0.08, 0.6)	0.131
LADS Anxiety score (max 18)	6.6 (4.2)	6.1 (2.6)	6.3 (2.8)	5.5 (2.1)	-0.4 (-3.3, 2.4)	0.763
LADS Depression score (max 18)	5.8 (3.5)	5.8 (2.3)	6.4 (1.8)	3.5 (3.5)	-0.2 (-2.7, 2.4)	0.901
Sleep disturbance (ESS score)	9.0 (5.7)	8.5 (7.6)	9.5 (8.3)	4.5 (2.1)	-0.1 (-4.2, 4.0)	0.961
REM Sleep Behaviour Disorder (RBDSQ) scale score	5.8 (3.4)	4.3 (2.5)	4.4 (2.8)	4.0 (0.0)	-1.2 (-3.6, 1.1)	0.307

Autonomic function: SCOPA total score	10.8 (6.9)	12.3 (7.4)	9.5 (4.8)	20.5 (9.2)	0.1 (-5.0, 5.3)	0.959
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SD = standard deviation; CI = confidence interval; MDS-UPDRS = Movement Disorder Society Unified Parkinson's Disease Rating Scale; PIGD = postural instability gait difficulty; MoCA= Montreal Cognitive Assessment; LADS = Leeds Anxiety and Depression Scale; ESS= Epworth Sleep Scale; RBDSQ = Rapid Eye Movement Sleep Behaviour Disorder Screening Questionnaire; SCOPA = Scales for Outcomes in Parkinson's disease.

^a *P* value of clinical features of *PRKN* and *PINK1* carriers together compared to non-carriers, excluding patients with dominant gene mutations. Adjusting for age at entry, gender, disease duration at entry/assessment and LEDD total, unless otherwise specified.

^b Adjusting for gender and disease duration at entry

^c Adjusting for gender and age at entry.

^d Adjusting for age, gender and LEDD total.

417 *Prevalence*

418 In the recent onset cohort (both young-onset and late-onset), the rate of pathogenic mutations
419 was 1.0% (17/1787). This is a large-scale cohort unselected for AAO, family history and genetic
420 status. From this, we can estimate the frequency of pathogenic mutations in the general UK
421 Parkinson's disease population. The crude prevalence rate of genetic forms of Parkinson's
422 disease is 951 per 100 000 (95% CI 892-1013, using the Poisson distribution). Age specific rates
423 are presented in Table 8. The age-standardised rate of genetic forms of Parkinson's disease was
424 708 per 100 000 (95% confidence interval 657-762 per 100 000), standardised to the mid-2016
425 Great Britain population. This provides an estimate of approximately 725 genetic Parkinson's
426 disease patients in a total of 102,403 patients in the UK currently living, using estimates from a
427 meta-analysis (Wickremaratchi *et al.*, 2009b) and the Office of National Statistics Great Britain
428 population estimates for mid-2016 (Office for National Statistics, 2017) assuming these genes do
429 not impact on survival (see Methods). A recent report from Parkinson's UK using primary care
430 diagnosis estimated a larger number of Parkinson's disease patients in the UK (145,519) in 2018
431 (Parkinson's UK, 2017). If this figure is more accurate, then the number of genetic Parkinson's
432 disease cases would be larger (estimated at 1030).

433

Table 8. Age specific and crude prevalence rate of genetic forms of Parkinson's disease, using data from **recent onset patients** only.

Age	Parkinson's disease genetic patients in cohort	Total number of Parkinson's disease patients in cohort (screened)	Age specific rates per 100,000 Parkinson's disease patients
0-29	0	0	0
30-39	1	11	9091
40-49	4	58	6897
50-59	4	219	1826
60-69	5	728	687
70-79	2	633	316
≥80	1	138	725
Total	17	1787	
Crude prevalence per 100,000 Parkinson's disease patients	951 (525-1442)		
Age adjusted prevalence per 100,000 Parkinson's disease patients*	708 (612-713)		

*Age distribution derived from age-specific Parkinson's disease rates (Wickremaratchi *et al.*, 2009a) applied to the UK mid-2016 population estimates (Office for National Statistics, 2017).

DISCUSSION

This study represents the largest study examining the rate of known Parkinson's disease gene mutations. We report an overall rate of mutations of 1.4% (29/2005), across both young-onset and late-onset patients. In combination with *GBA* gene analysis in the same cohort (Malek *et al.*, 2018), our results suggest that up to 10% of Parkinson's disease patients carry a genetic variant that could potentially be targeted by new drug therapies. For instance, G2019S and other mutations in the *LRRK2* gene have been shown to increase kinase activity, and *LRRK2* kinase inhibitors that counteract this activity are currently being tested in phase 1 clinical trials as a potential therapeutic target for Parkinson's disease (reviewed in (Atashrazm and Dzamko, 2016; Taymans and Greggio, 2016; Alessi and Sammler, 2018)).

The strengths of this study lie in the relatively unbiased, population-based patient ascertainment. This increases the generalisability of our findings, specifically the prevalence estimates of Parkinson's disease patients carrying pathogenic mutations based on the incident recent-onset cohort. A further strength of this study is inclusion of both early and late-onset patients, where previous genetic studies have focused on early-onset patients.

Firstly, this has enabled us to more accurately estimate the prevalence of mutations in the general Parkinson's disease UK population, assuming there are no survival effects, rather than just in the subset of early-onset patients. We show clearly that *LRRK2* mutations are present at a significant rate in patients with onset under 50 years (2.2%), and that *SNCA* mutations are present in 1.5% of patients with a strong family history of Parkinson's disease (2 or more additional family members affected).

Secondly, our findings suggest that there may be other high-risk genes that have not yet been identified. In particular, further efforts in gene discovery can focus on the substantial proportion of patients with very early onset or who have a large family history in which no known pathogenic mutations have been identified.

Thirdly, our findings have implications for genetic testing. Although further work is needed to confirm some results, our data suggest that *LRRK2* mutations are common in young-onset Parkinson's disease (2.2%) and should be more regularly tested with appropriate genetic counselling. Additionally, our results highlight the importance of systematically screening for

copy number variants in *PRKN*, *PINK1* and *SNCA*, as these are common variants and may be missed with sequencing methods such as exome sequencing.

Finally, we show there are systematic clinical differences between recessive gene mutation carriers compared to early-onset non-carriers. *PRKN* and *PINK1* carriers have more postural problems at diagnosis and better cognition than other early onset patients, even after adjusting for age, disease duration, gender and LEDD.

LRRK2 and *SNCA*

Mutations in *LRRK2* (PARK8, dardarin) were first identified in autosomal dominant, mostly late-onset families with Parkinson's disease (Funayama *et al.*, 2002; Paisán-Ruiz *et al.*, 2004; Zimprich *et al.*, 2004). The reported frequency of *LRRK2* mutations varies widely; mutations are more common in familial Parkinson's disease (5-6%) (Di Fonzo *et al.*, 2005; Nichols *et al.*, 2005) than in sporadic disease (~1%) (Gilks *et al.*, 2005; Hernandez *et al.*, 2005). However the frequency of mutations also differs according to population, and the G2019S mutation may be more common in Southern Europe than in Northern Europe (Bonifati, 2007). The rate of mutations is particularly high in Ashkenazi Jewish (up to 28%) and North African patients (up to 41%) (Lesage *et al.*, 2005, 2006; Ozelius *et al.*, 2006; Williams-Gray *et al.*, 2006; Healy *et al.*, 2008; Puschmann, 2013). We found that *LRRK2* mutations were present at a rate of 0.9% overall, most commonly the G2019S mutation (0.8%). Our findings are comparable with a previous community-based cohort in the UK (Williams-Gray *et al.*, 2006) and other Caucasian North American and UK cohorts with estimates between 0.4 and 1.7% (Deng *et al.*, 2005; Farrer *et al.*, 2005; Hernandez *et al.*, 2005; Zabetian *et al.*, 2005). Our results are also in accordance with a combined analysis of previous G2019S studies which estimated a mean prevalence of 0.9%, although this was across different populations (Williams-Gray *et al.*, 2006).

R1441C mutations were present in 0.4 % of young-onset and familial patients. This is in keeping with other studies showing the rarity of *LRRK2* R1441C mutations in Caucasian populations, with previous studies reporting frequencies between 0% and 0.3% (Zabetian *et al.*, 2005; Pankratz *et al.*, 2006; Möller *et al.*, 2008). To our knowledge, this study is the first to systematically screen and report on the prevalence of R1441C mutations in early onset and/or familial Parkinson's disease in the UK.

Almost half of our *LRRK2* carriers did not report a family history of Parkinson's disease. Although the first reports of *LRRK2* mutations were in families with multiple affected members, later studies have shown that a large proportion of *LRRK2* carriers do not have other family members affected by Parkinson's disease (Gilks *et al.*, 2005; Ozelius *et al.*, 2006). This is likely due to the reduced penetrance of *LRRK2* mutations. The penetrance of both the G2019S and R1441C mutations is incomplete (24% to 42% up to age 80 for G2019S), strongly age-dependent and increases in a linear fashion (Clark *et al.*, 2006b; Ozelius *et al.*, 2006; Healy *et al.*, 2008; Lee *et al.*, 2017). As the population ages, it is likely that increasing numbers of *LRRK2* relatives will develop Parkinson's disease as a result of *LRRK2* mutations, and the prevalence of this form of Parkinson's disease will increase in the UK.

As reported in some previous studies (Di Fonzo *et al.*, 2005; Gilks *et al.*, 2005; Kay *et al.*, 2006; Haugarvoll *et al.*, 2008), we found that *LRRK2* carriers presented with a range of AAOs (35 to 79 years). *LRRK2* mutations were also more common in young-onset patients (2.2%) than in late-onset patients (0.5%). However, a combined analysis of all studies in MDSGene showed that the majority (94%) of *LRRK2* carriers have late AAO (Trinh *et al.*, 2018). Our findings do not support this pattern and further work must be done to clarify this. It may be that studies included in MDSGene were more likely to screen late-onset patients and not young-onset patients for *LRRK2*. This is difficult to assess as MDSGene only compares characteristics of mutation carriers and not non-carriers. Our findings may have implications for genetic testing where, in the UK, *LRRK2* testing is recommended for late-onset patients with a family history of Parkinson's disease. We suggest that *LRRK2* should be tested more frequently in young-onset patients, even those without a family history of Parkinson's disease, however additional studies in both young-onset and late-onset patients are needed.

We report two distinct G2019S haplotypes, in keeping with previous studies showing the mutation has been found in 3 major haplotypes. Haplotype 1 is the most common, present in European, North American populations of European, Arab and Jewish origin (Goldwurm *et al.*, 2005; Kachergus *et al.*, 2005; Lesage *et al.*, 2006, 2010; Zabetian *et al.*, 2006a). Haplotype 2 has been reported in North American families of European origin (Zabetian *et al.*, 2006a) and French families (Lesage *et al.*, 2010). The third haplotype has been found in Japanese patients (Zabetian *et al.*, 2006b). We show the presence of both haplotype 1 and haplotype 2 in our patients. These

distinct haplotypes suggest there have been at least 2 independent founding events from which the G2019S mutation arose, one likely from an ancient Middle Eastern founder (Ozelius *et al.*, 2006; Zabetian *et al.*, 2006a; Lesage *et al.*, 2010).

The R1441C mutation in *LRRK2* has also been found on at least two distinct haplotypes. The first haplotype is reported in a North American family originating from England (Wszolek *et al.*, 1995; Zimprich *et al.*, 2004) and in Flemish-Belgian families (Haugarvoll *et al.*, 2008; Nuytemans *et al.*, 2008), suggesting a common founder. The second haplotype is present in Italian, German, Spanish, North American and Iranian patients (Zimprich *et al.*, 2004; Haugarvoll *et al.*, 2008; Shojaaee *et al.*, 2009). These haplotypes suggest that the R1441C mutation also arose in two independent events/founders, rather than a single ancient founder. Our constructed R1441C haplotypes were consistent with previous reports but we were unable to distinguish between the two different haplotypes.

We did not find any differences in motor or non-motor features between *LRRK2* carriers and non-carriers. Several studies and reviews suggest that *LRRK2* mutations are associated with a more benign disease course, less severe clinical symptoms (Nichols *et al.*, 2005), lower risk of cognitive impairment and better cognitive performance (Healy *et al.*, 2008; Srivatsal *et al.*, 2015; Kasten *et al.*, 2017). The MDSGene systematic review also suggested that *LRRK2* carriers have a good response to L-dopa, late AAO and absence of atypical signs (Trinh *et al.*, 2018). However other studies have not confirmed these findings (Lesage *et al.*, 2005; Haugarvoll *et al.*, 2008; Healy *et al.*, 2008; Alcalay *et al.*, 2010b; Belarbi *et al.*, 2010; Ben Sassi *et al.*, 2012; Puschmann, 2013; De Rosa *et al.*, 2014; Estanga *et al.*, 2014).

SNCA mutations were first identified in large Parkinson's disease families with an autosomal dominant pattern of inheritance (Polymeropoulos *et al.*, 1997; Muentner *et al.*, 1998; Singleton *et al.*, 2003). *SNCA* mutations are rare in studies of Caucasian patients (Scott *et al.*, 1999; Berg *et al.*, 2005; Nuytemans *et al.*, 2009). We found one patient carrying a heterozygous duplication, comprising 1.5% of patients reporting 2 or more additional family members affected by Parkinson's disease. This is in line with previous studies reporting a mutation prevalence of 1.7% to 5.8% in familial Parkinson's disease patients (Farrer *et al.*, 2004; Ibáñez *et al.*, 2004; Nishioka *et al.*, 2009; Bozi *et al.*, 2014).

It has previously been reported that *SNCA* mutation carriers have more frequent and more severe dementia, rapid progression, hallucinations and autonomic dysfunction (Muentert *et al.*, 1998; Farrer *et al.*, 2004; Fuchs *et al.*, 2007; Ahn *et al.*, 2008; Nishioka *et al.*, 2009; Puschmann, 2013; Bonifati, 2014; Kasten *et al.*, 2017; Schneider and Alcalay, 2017). *SNCA* triplications cause a more severe phenotype while duplications tend to cause more 'typical' Parkinson's disease (Chartier-Harlin *et al.*, 2004; Ibáñez *et al.*, 2004; Hernandez *et al.*, 2016). We were not able to compare clinical features this in our cohort due to the rarity of *SNCA* mutations.

Our cohort represents the largest UK-based series of *LRRK2* and *SNCA* carriers and non-carriers identified from the same unselected population, including both early and late onset patients. In line with many previous studies, our findings suggest that Parkinson's disease caused by *LRRK2* mutations duplications is clinically indistinguishable from sporadic disease.

Young-onset Parkinson's disease

We found pathogenic mutations in 6.3% (19/302) of young-onset patients, including mutations in both dominant and recessive genes. These are comparable to the frequencies previously reported in other young-onset cohorts (Alcalay *et al.*, 2010a; Kilariski *et al.*, 2012; Kim and Alcalay, 2017). In accordance with previous studies (Alcalay *et al.*, 2010a; Marder *et al.*, 2010), we show that mutations were more common in patients with earlier onset.

We identified compound heterozygous *PRKN* mutations in 2.6% of young-onset patients. While this is lower than other prevalence estimates in Caucasian populations (Abbas *et al.*, 1999; Lücking *et al.*, 2000; Lohmann *et al.*, 2003; Periquet *et al.*, 2003), our findings are in accordance with a previous UK community-based study which found that *PRKN* mutations accounted for 3.7% of patients with onset under 45 years (Kilariski *et al.*, 2012).

We also identified 3% patients carrying single heterozygous pathogenic mutations in *PRKN* and *PINK1*. Our frequency of single heterozygous carriers is similar to what has been reported in other studies, although these include varying methods for identifying copy number variants (Klein *et al.*, 2007; Marder *et al.*, 2010).

Previous studies suggest that *PRKN* mutations are more common in familial patients (Alcalay *et al.*, 2010a). We found a trend for *PRKN* mutations to be more common in familial (4.2%) than in

sporadic patients (1.9%), although not significantly different. However, 20% of patients with 2 additional family members affected carried *PRKN* mutations.

We found evidence for a shared haplotype for the P113Xfs mutation in five carriers across three markers spanning 242 kB. Our analysis does not include genotyping of microsatellite markers which are needed for more detailed haplotype analysis. However our findings are consistent with previous evidence showing that point mutations have shared haplotypes and may originate from a common founder (Farrer *et al.*, 2001; Periquet *et al.*, 2001).

PINK1 mutation carriers were present in 0.7% of young-onset patients. This is comparable to the rate reported in a previous community-based study (Kilarski *et al.*, 2012). Mutations are more common in Asian and Italian patients (Hatano *et al.*, 2004; Valente *et al.*, 2004; Bonifati *et al.*, 2005; Li *et al.*, 2005; Tan *et al.*, 2006), reflecting population-specific allele frequencies. Our findings are consistent with the low prevalence estimates in Northern Europe and North American patients (Healy *et al.*, 2004; Rogaeva *et al.*, 2004). However contrary to previous reports (Kilarski *et al.*, 2012), we did not find that mutations were more frequent in patients with a family history of Parkinson's disease (1.1%) compared to sporadic patients (0.5%). This may be due to the small number of *PINK1* carriers in our cohort.

After controlling for age and disease duration, we found that *PRKN* and *PINK1* carriers had earlier AAO, reported more postural symptoms at diagnosis and had better cognition compared to other young-onset patients. This is consistent with previous studies showing that *PRKN* and *PINK1* mutations are generally associated with slower disease progression and less cognitive impairment (Valente *et al.*, 2001, 2004, Lohmann *et al.*, 2003, 2012; Bonifati *et al.*, 2005; Tan *et al.*, 2006; Alcalay *et al.*, 2014; Bonifati, 2014; Kasten *et al.*, 2017; Kim and Alcalay, 2017). Some studies have suggested that atypical features, such as dystonia, and psychiatric symptoms may be more common in *PINK1* and *PRKN* carriers (Bonifati *et al.*, 2005; Kasten *et al.*, 2017; Koros *et al.*, 2017), however we did not find evidence to support this; there is also substantial variability of the frequency of these symptoms in previous reports (Kasten *et al.*, 2017). Our findings are in line with a recent MDSGene systematic review, which suggested that recessive gene mutation carriers have less common cognitive decline, good treatment response and otherwise clinically typical disease (Kasten *et al.*, 2018). While a few conflicting reports suggest there are no clinical differences between *PRKN* carriers and non-carriers (Lohmann *et al.*, 2009),

our findings in a large population-based study definitively show that there are clinical differences between mutation carriers and non-carriers. This may be associated with the lack of Lewy body pathology in the brain at post-mortem (Takahashi H, Ohama E, 1994; Mori *et al.*, 1998), although there are small numbers of *PRKN* cases with pathological data and there is variability in findings (Farrer *et al.*, 2001; Schneider and Alcalay, 2017).

Limitations

Our cohort was predominantly Caucasian and no pathogenic mutations were identified in non-Caucasian groups. Therefore, the estimated rate of mutations has limited application in other populations. Further large-scale studies are needed to establish mutation prevalence in other ethnic groups. Our results are also limited by the lack of complete screening; exome sequencing, MLPA and *PRKN* and *PINK1* sequencing of all patients was not feasible due to cost limitations and the size of the cohort. Recessive gene mutations are rare in patients with older onset (Alcalay *et al.*, 2010a; Kilariski *et al.*, 2012), however *PRKN* mutations have been identified in late-onset patients with onset up to 78 years (Foroud *et al.*, 2003; Klein *et al.*, 2003). Therefore, there may have been a small number of mutation carriers that were not detected with our screening methods. Our data therefore represents a minimal estimate of the frequency of genetic mutations and true numbers may be slightly higher. Our genetic rates are based on both incident and prevalent cases. We have assumed that survival and hence prevalence is not influenced by these genes but if some genes e.g. *PRKN* and *PINK1* are associated with better survival then we may have under-estimated the number of cases in the general population.

A further limitation is that, while this is a large cohort study, the rarity of pathogenic mutations means that our group difference comparisons may be under-powered to detect modest phenotypic differences. Finally, our cohort is likely to still have some biases in it, given we did not undertake a rigorous community based study collecting all cases of the condition.

Conclusions

We show that Mendelian gene mutations are a rare but significant cause of Parkinson's disease. Patients with *PRKN* and *PINK1* mutations differ from other early onset patients in cognition and postural symptoms. In combination with estimates of *GBA* mutation prevalence, this large-scale, relatively unbiased study suggests that up to 10% of Parkinson's disease patients carry known

642 genetic variants that could be targeted by new drug therapies in clinical trials and future
643 treatment.

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References

- Abbas N, Lücking CB, Ricard S, Dürr A, Bonifati V, De Michele G, et al. A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. *Hum. Mol. Genet.* 1999; 8: 567–74.
- Ahn T-B, Kim SY, Kim JY, Park S-S, Lee DS, Min HJ, et al. Alpha-synuclein gene duplication is present in sporadic Parkinson disease. *Neurology* 2008; 71: 1294; author reply 1294.
- Alcalay RN, Caccappolo E, Mejia-Santana H, Tang M-X, Rosado L, Orbe Reilly M, et al. Cognitive performance of GBA mutation carriers with early-onset PD: the CORE-PD study. *Neurology* 2012; 78: 1434–40.
- Alcalay RN, Caccappolo E, Mejia-Santana H, Tang MX, Rosado L, Orbe Reilly M, et al. Cognitive and motor function in long-duration PARKIN-associated Parkinson disease. *JAMA Neurol.* 2014; 71: 62–7.
- Alcalay RN, Caccappolo E, Mejia-Santana H, Tang MX, Rosado L, Ross BM, et al. Frequency of Known Mutations in Early-Onset Parkinson Disease. *Arch. Neurol.* 2010a; 67: 1116–1122.
- Alcalay RN, Mejia-Santana H, Tang MX, Rakitin B, Rosado L, Ross B, et al. Self-report of cognitive impairment and Mini-Mental State Exam performance in PRKN, LRRK2, and GBA carriers with early onset Parkinson's disease. *J Clin Exp Neuropsychol.* 2010b; 32: 775–779.
- Alessi DR, Sammler E. LRRK2 kinase in Parkinson's disease. *Science* 2018; 360
- Atashrazm F, Dzamko N. LRRK2 inhibitors and their potential in the treatment of Parkinson's disease: current perspectives. *Clin. Pharmacol.* 2016; 8: 177–189.
- Auton A, Abecasis GR, Altshuler DM, Durbin RM, Bentley DR, Chakravarti A, et al. A global reference for human genetic variation. *Nature* 2015; 526: 68–74.
- Belarbi S, Hecham N, Lesage S, Kediha MI, Smail N, Benhassine T, et al. LRRK2 G2019S mutation in Parkinson's disease: A neuropsychological and neuropsychiatric study in a large Algerian cohort. *Park. Relat. Disord.* 2010; 16: 676–679.
- Berg D, Niwar M, Maass S, Zimprich A, Möller JC, Wuellner U, et al. Alpha-synuclein and Parkinson's disease: Implications from the screening of more than 1,900 patients. *Mov. Disord.* 2005; 20: 1191–1194.
- Bonifati V. LRRK2 Low-penetrance Mutations (Gly2019Ser) and Risk Alleles (Gly2385Arg)—Linking Familial and Sporadic Parkinson's Disease. *Neurochem. Res.* 2007; 32: 1700–1708.
- Bonifati V. Genetics of Parkinson's disease – state of the art, 2013. *Parkinsonism Relat. Disord.* 2014; 20S1: S23–S28.
- Bonifati V, Rohe CF, Breedveld GJ, Fabrizio E, Mari M De, Tassorelli C, et al. Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes. *Neurology* 2005; 65: 87–95.
- Bozi M, Papadimitriou D, Antonellou R, Moraitou M, Maniati M, Vassilatis DK, et al. Genetic assessment of familial and early-onset Parkinson's disease in a Greek population. *Eur. J. Neurol.* 2014; 21: 963–968.
- Chartier-Harlin M-CJK, Roumier C, Mouroux V, Douay X, Lincoln S, Levecque C, et al. α -

- synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 2004; 364: 1169–1171.
- Cilia R, Tunesi S, Marotta G, Cereda E, Tesei S, Zecchinelli A, et al. Survival and dementia in GBA -associated Parkinson Disease : the mutation matters . : 1–38.
- Clark LN, Ross BM, Wang Y, Mejia-Santana H, Harris J, Louis ED, et al. Mutations in the glucocerebrosidase gene are associated with early-onset Parkinson disease. *Neurology* 2007; 69: 1270–1277.
- Clark LN, Wang Y, Karlins E, Saito L, Harris J, Louis ED, et al. Frequency of LRRK2 mutations in early- and late-onset Parkinson disease. 2006a
- Clark LN, Wang Y, Karlins E, Saito L, Mejia-Santana H, Harris J, et al. Frequency of LRRK2 mutations in early- and late-onset Parkinson disease. *Neurology* 2006b; 67: 1786–1791.
- Crosiers D, Verstraeten A, Wauters E, Engelborghs S, Peeters K, Mattheijssens M, et al. Mutations in glucocerebrosidase are a major genetic risk factor for Parkinson's disease and increase susceptibility to dementia in a Flanders-Belgian cohort. *Neurosci. Lett.* 2016; 629: 160–164.
- Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. *Nat. Genet.* 2016; 48: 1284–1287.
- Davis A a., Andruska KM, Benitez B a., Racette B a., Perlmutter JS, Cruchaga C. Variants in GBA, SNCA, and MAPT Influence Parkinson Disease Risk, Age at Onset, and Progression. *Neurobiol. Aging* 2016; 37: 209.e1-209.e7.
- Deng H, Le W, Guo Y, Hunter CB, Xie W, Jankovic J. Genetic and clinical identification of Parkinson's disease patients with LRRK2 G2019S mutation. *Ann. Neurol.* 2005; 57: 933–934.
- Estanga A, Rodriguez-Oroz MC, Ruiz-Martinez J, Barandiaran M, Gorostidi A, Bergareche A, et al. Cognitive dysfunction in Parkinson's disease related to the R1441G mutation in LRRK2. *Park. Relat. Disord.* 2014; 20: 1097–1100.
- Farrer M, Chan P, Chen R, Tan L, Lincoln S, Hernandez D, et al. Lewy bodies and parkinsonism in families with parkin mutations. *Ann. Neurol.* 2001; 50: 293–300.
- Farrer M, Kachergus J, Forno L, Lincoln S, Wang DS, Hulihan M, et al. Comparison of Kindreds with Parkinsonism and α -Synuclein Genomic Multiplications. *Ann. Neurol.* 2004; 55: 174–179.
- Farrer M, Stone J, Mata IF, Lincoln S, Kachergus J, Hulihan M, et al. LRRK2 mutations in Parkinson disease. *Neurology* 2005; 65: 738–740.
- Di Fonzo A, Rohé CF, Ferreira J, Chien HF, Vacca L, Stocchi F, et al. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet* 2005; 365: 412–415.
- Foroud T, Uniacke SK, Liu L, Pankratz N, Rudolph a, Halter C, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. *Neurology* 2003; 60: 796–801.
- Fuchs J, Nilsson C, Kachergus J, Munz M, Larsson EM, Schüle B, et al. Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. *Neurology* 2007; 68: 916–922.
- Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's Disease (PARK8) maps to chromosome 12p11.2-q13.1. *Ann. Neurol.* 2002; 51: 296–301.

- 747 Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, et al. A common LRRK2
748 mutation in idiopathic Parkinson's disease. *Lancet* 2005; 365: 415–416.
- 749 Goldwurm S, Fonzo A Di, Simons EJ, Rohe CF, Zini M, Canesi M, et al. The G6055A (G2019S)
750 mutation in LRRK2 is frequent in both early and late onset Parkinson's disease and originates
751 from a common ancestor. *J. Med. Genet.* 2005; 42: e65–e65.
- 752 Golub Y, Berg D, Calne DB, Pfeiffer RF, Uitti RJ, Stoessl a J, et al. Genetic factors influencing
753 age at onset in LRRK2-linked Parkinson disease. *Parkinsonism Relat. Disord.* 2009; 15: 539–
754 541.
- 755 Hatano Y, Li Y, Sato K, Asakawa S, Yamamura Y, Tomiyama H, et al. Novel PINK1 mutations
756 in early-onset parkinsonism. *Ann. Neurol.* 2004; 56: 424–427.
- 757 Haugarvoll K, Rademakers R, Kachergus JM, Nuytemans K, Ross OA, Gibson JM, et al. Lrrk2
758 R1441C parkinsonism is clinically similar to sporadic Parkinson disease. *Neurology* 2008; 70:
759 1456–1460.
- 760 Healy DG, Abou-Sleiman PM, Gibson JM, Ross OA, Jain S, Gandhi S, et al. PINK1 (PARK6)
761 associated Parkinson disease in Ireland. *Neurology* 2004; 63: 1486–1488.
- 762 Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype,
763 genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-
764 control study. *Lancet Neurol.* 2008; 7: 583–590.
- 765 Hernandez D, Paisan Ruiz C, Crawley A, Malkani R, Werner J, Gwinn-Hardy K, et al. The
766 dardarin G2019S mutation is a common cause of Parkinson's disease but not other
767 neurodegenerative diseases. *Neurosci. Lett.* 2005; 389: 137–139.
- 768 Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: Mendelian versus non-
769 Mendelian inheritance. *J. Neurochem.* 2016; 139: 59–74.
- 770 Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body
771 Parkinson's disease. 2001
- 772 Ibáñez P, Bonnet a-M, Débarges B, Lohmann E, Tison F, Pollak P, et al. Causal relation
773 between alpha-synuclein gene duplication and familial Parkinson's disease. *Lancet* 2004; 364:
774 1169–1171.
- 775 Kachergus J, Mata IF, Hulihan M, Taylor JP, Lincoln S, Aasly J, et al. Identification of a novel
776 LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder
777 across European populations. *Am. J. Hum. Genet.* 2005; 76: 672–80.
- 778 Kasten M, Hartmann C, Hampf J, Schaake S, Westenberger A, Vollstedt EJ, et al. Genotype-
779 Phenotype Relations for the Parkinson's Disease Genes Parkin, PINK1, DJ1: MDSGene
780 Systematic Review. *Mov. Disord.* 2018; 33: 730–741.
- 781 Kasten M, Marras C, Klein C. Nonmotor Signs in Genetic Forms of Parkinson's Disease
782 [Internet]. 1st ed. Elsevier Inc.; 2017. Available from:
783 <http://dx.doi.org/10.1016/bs.irm.2017.05.030>
- 784 Kay DM, Zabetian CP, Factor SA, Nut JG, Samii A, Griffith A, et al. Parkinson's disease and
785 LRRK2: Frequency of a common mutation in U.S. movement disorder clinics. *Mov. Disord.*
786 2006; 21: 519–523.
- 787 Kilarski LL, Pearson JP, Newsway V, Majounie E, Knipe MDW, Misbahuddin A, et al.

- 788 Systematic review and UK-based study of PARK2 (parkin), PINK1, PARK7 (DJ-1) and LRRK2
789 in early-onset Parkinson's disease. *Mov. Disord.* 2012; 27: 1522–9.
- 790 Kim CY, Alcalay RN. Genetic Forms of Parkinson ' s Disease. 2017
- 791 Klebe S, Golmard J-L, Nalls M a., Saad M, Singleton a. B, Bras JM, et al. The Val158Met
792 COMT polymorphism is a modifier of the age at onset in Parkinson's disease with a sexual
793 dimorphism. *J. Neurol. Neurosurg. Psychiatry* 2013; 84: 666–673.
- 794 Klein C, Hedrich K, Wellenbrock C, Kann M, Harris J, Marder K, et al. Frequency of parkin
795 mutations in late-onset Parkinson's disease. *Ann. Neurol.* 2003; 54: 415–416.
- 796 Klein C, Lohmann-Hedrich K, Rogaeva E, Schlossmacher MG, Lang AE. Deciphering the role
797 of heterozygous mutations in genes associated with parkinsonism. *Lancet Neurol.* 2007; 6: 652–
798 662.
- 799 Koros C, Simitsi A, Stefanis L. Genetics of Parkinson's Disease: Genotype–Phenotype
800 Correlations [Internet]. 1st ed. Elsevier Inc.; 2017. Available from:
801 <http://dx.doi.org/10.1016/bs.irm.2017.01.009>
- 802 Kwok JBJ, Teber ET, Loy C, Hallupp M, Nicholson G, Mellick GD, et al. Tau Haplotypes
803 Regulate Transcription and Are Associated with Parkinson's Disease. *Ann. Neurol.* 2004; 55:
804 329–334.
- 805 Lee AJ, Wang Y, Alcalay RN, Mejia-Santana H, Saunders-Pullman R, Bressman S, et al.
806 Penetrance estimate of LRRK2 p.G2019S mutation in individuals of non-Ashkenazi Jewish
807 ancestry. *Mov. Disord.* 2017; 32: 1432–1438.
- 808 Lesage S, Brice A. Role of mendelian genes in 'sporadic' Parkinson's disease. *Parkinsonism*
809 *Relat. Disord.* 2012; 18 Suppl 1: S66-70.
- 810 Lesage S, Dürr A, Tazir M, Lohmann E, Leutenegger A-L, Janin S, et al. *LRRK2* G2019S as a
811 Cause of Parkinson's Disease in North African Arabs. *N. Engl. J. Med.* 2006; 354: 422–423.
- 812 Lesage S, Ibanez P, Lohmann E, Pollak P, Tison F, Tazir M, et al. G2019S *LRRK2* mutation in
813 French and North African families with Parkinson's disease. *Ann. Neurol.* 2005; 58: 784–787.
- 814 Lesage S, Patin E, Condroyer C, Leutenegger AL, Lohmann E, Giladi N, et al. Parkinson's
815 disease-related *LRRK2* G2019S mutation results from independent mutational events in humans.
816 *Hum. Mol. Genet.* 2010; 19: 1998–2004.
- 817 Li Y, Tomiyama H, Sato K, Hatano Y, Yoshino H, Atsumi M, et al. Clinicogenetic study of
818 *PINK1* mutations in autosomal recessive early-onset parkinsonism. *Neurology* 2005; 64: 1955–
819 1957.
- 820 Lill CM, Mashychev A, Hartmann C, Lohmann K, Marras C, Lang AE, et al. Launching the
821 movement disorders society genetic mutation database (MDSGene). *Mov. Disord.* 2016; 31:
822 607–609.
- 823 Lohmann E, Dursun B, Lesage S, Hanagasi HA, Sevinc G, Honore A, et al. Genetic bases and
824 phenotypes of autosomal recessive Parkinson disease in a Turkish population. *Eur. J. Neurol.*
825 2012; 19: 769–775.
- 826 Lohmann E, Periquet M, Bonifati V, Wood NW, De Michele G, Bonnet AM, et al. How much
827 phenotypic variation can be attributed to parkin genotype? *Ann. Neurol.* 2003; 54: 176–185.
- 828 Lohmann E, Thobois S, Lesage S, Broussolle E, Du Montcel ST, Ribeiro MJ, et al. A

multidisciplinary study of patients with early-onset PD with and without parkin mutations. *Neurology* 2009; 72: 110–116.

Lubbe S, Morris HR. Recent advances in Parkinson's disease genetics. *J. Neurol.* 2014; 261: 259–266.

Lücking CB, Dürr A, Bonifati V, Vaughan J, De Michele G, Gasser T, et al. Association between early-onset Parkinson's disease and Mutations in the Parkin Gene. October 2000

Malek N, Swallow DM a., Grosset K a., Lawton M a., Smith CR, Bajaj NP, et al. Olfaction in *Parkin* single and compound heterozygotes in a cohort of young onset Parkinson's disease patients. *Acta Neurol. Scand.* 2015a: n/a-n/a.

Malek N, Swallow DMA, Grosset KA, Lawton MA, Marrinan SL, Lehn AC, et al. Tracking Parkinson's: Study Design and Baseline Patient Data. *J. Parkinsons. Dis.* 2015b; 5: 947–959.

Malek N, Weil RS, Bresner C, Lawton MA, Grosset KA, Tan M, et al. Features of *GBA* - associated Parkinson's disease at presentation in the UK *Tracking Parkinson's* study. *J. Neurol. Neurosurg. Psychiatry* 2018: jnnp-2017-317348.

Marder K, M-x T, Mejia-Santana H, Rosada L, Louis E. Predictors of parkin mutations in early onset parkinson disease: the CORE-PD study. *Arch. Neurol.* 2010; 67: 731–738.

Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP, Koller WC, et al. Association of single-nucleotide polymorphisms of the tau gene with late-onset Parkinson disease. *JAMA* 2001; 286: 2245–2250.

Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Chen-Plotkin A, Van Deerlin VM, et al. *GBA* Variants are associated with a distinct pattern of cognitive deficits in Parkinson's disease. *Mov. Disord.* 2015; 00: n/a-n/a.

Möller JC, Rissling I, Mylius V, Höft C, Eggert KM, Oertel WH. The prevalence of the G2019S and R1441C/G/H mutations in LRRK2 in German patients with Parkinson's disease. *Eur. J. Neurol.* 2008; 15: 743–745.

Mori H, Kondo T, Yokochi M, Matsumine H, Nakagawa-Hattori Y, Miyake T, et al. Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. *Neurology* 1998; 51: 890–892.

Muenter MD, Forno LS, Hornykiewicz O, Kish SJ, Maraganore DM, Caselli RJ, et al. Hereditary form of parkinsonism-dementia. *Ann. Neurol.* 1998; 43: 768–781.

Nichols WC, Pankratz N, Hernandez D, Paisán-Ruiz C, Jain S, Halter CA, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. *Lancet* 2005; 365: 410–412.

Nishioka K, Ross OA, Ishii K, Kachergus JM, Ishiwata K, Kitagawa M, et al. Expanding the clinical phenotype of SNCA duplication carriers. *Mov. Disord.* 2009; 24: 1811–1819.

Nuytemans K, Meeus B, Crosiers D, Brouwers N, Goossens D, Engelborghs S, et al. Relative contribution of simple mutations vs. copy number variations in five Parkinson disease genes in the Belgian population. *Hum. Mutat.* 2009; 30: 1054–1061.

Nuytemans K, Rademakers R, Theuns J, Pals P, Engelborghs S, Pickut B, et al. Founder mutation p.R1441C in the leucine-rich repeat kinase 2 gene in Belgian Parkinson's disease patients. *Eur. J. Hum. Genet.* 2008; 16: 471–479.

Office for National Statistics. Estimates of the population for the UK, England and Wales, Scotland and Northern Ireland [Internet]. 2017. Available from: <https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/datasets/populationestimatesforukenglandandwalesscotlandandnorthernireland>

Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligtisch A, Tagliati M, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N. Engl. J. Med.* 2006; 354: 424–425.

Paisán-Ruiz C, Jain S, Evans EW, Gilks WP, Simón J, Van Der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* 2004; 44: 595–600.

Pankratz N, Pauciulo MW, Elsaesser VE, Marek DK, Halter CA, Rudolph A, et al. Mutations in LRRK2 other than G2019S are rare in a north-American based sample of familial Parkinson's disease. *Mov. Disord.* 2006; 21: 2257–2260.

Parkinson's UK. The incidence and prevalence of Parkinson's in the UK. Results from the Clinical Practice Research Datalink Reference Report [Internet]. 2017 Available from: [https://www.parkinsons.org.uk/sites/default/files/2018-01/Prevalence Incidence Report Latest_Public_2.pdf](https://www.parkinsons.org.uk/sites/default/files/2018-01/Prevalence%20Incidence%20Report%20Latest_Public_2.pdf)

Periquet M, Latouche M, Lohmann E, Rawal N, De Michele G, Ricard S, et al. Parkin mutations are frequent in patients with isolated early-onset parkinsonism. *Brain* 2003; 126: 1271–1278.

Periquet M, Lücking C, Vaughan J, Bonifati V, Dürr a, De Michele G, et al. Origin of the mutations in the parkin gene in Europe: exon rearrangements are independent recurrent events, whereas point mutations may result from Founder effects. *Am. J. Hum. Genet.* 2001; 68: 617–626.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the Alpha-Synuclein Gene Identified in Families with Parkinson's Disease. *Science* (80-.). 1997; 276: 2045–2047.

Puschmann A. Monogenic Parkinson's disease and parkinsonism: Clinical phenotypes and frequencies of known mutations. *Parkinsonism Relat. Disord.* 2013; 19: 407–415.

Rogaeva E, Johnson J, Lang AE, Gulick C, Gwinn-Hardy K, Kawarai T, et al. Analysis of the PINK1 Gene in a Large Cohort of Cases With Parkinson Disease [Internet]. *Arch. Neurol.* 2004; 61 Available from: <http://archneur.jamanetwork.com/article.aspx?doi=10.1001/archneur.61.12.1898>

De Rosa A, De Michele G, Guacci A, Carbone R, Lieto M, Peluso S, et al. Genetic screening for the LRRK2 R1441C and G2019S mutations in parkinsonian patients from campania. *J. Parkinsons. Dis.* 2014; 4: 123–128.

Sanchez-Contreras M, Heckman MG, Tacik P, Diehl N, Brown PH, Soto-Ortolaza AI, et al. Study of LRRK2 variation in tauopathy: Progressive supranuclear palsy and corticobasal degeneration. *Mov. Disord.* 2017; 32: 115–123.

Ben Sassi S, Nabli F, Hentati E, Nahdi H, Trabelsi M, Ben Ayed H, et al. Cognitive dysfunction in Tunisian LRRK2 associated Parkinson's disease. *Park. Relat. Disord.* 2012; 18: 243–246.

Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with parkinsonism: Review of the literature. *Mov. Disord.* 2017; 32: 1504–1523.

- 912 Scott WK, Yamaoka LH, Stajich JM, Scott BL, Vance JM, Roses AD, et al. The alpha-synuclein
913 gene is not a major risk factor in familial Parkinson disease. *Neurogenetics* 1999; 2: 191–192.
- 914 Shojae S, Sina F, Farboodi N, Fazlali Z, Ghazavi F, Ghorashi SA, et al. A clinic-based
915 screening of mutations in exons 31, 34, 35, 41, and 48 of LRRK2 in Iranian Parkinson's disease
916 patients. *Mov. Disord.* 2009; 24: 1023–1027.
- 917 Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, et al. Alpha-Synuclein
918 Locus Triplication Causes Parkinson's Disease. *Science* (80-.). 2003; 302: 841–841.
- 919 Srivatsal S, Cholerton B, Leverenz JB, Wszolek ZK, Uitti RJ, Dickson DW, et al. Cognitive
920 profile of LRRK2-related Parkinson's disease. *Mov. Disord.* 2015; 30: 728–733.
- 921 Takahashi H, Ohama E SS et al. Familial juvenile parkinsonism: Clinical and pathologic study in
922 a family. *Neurology* 1994; 44: 437–441.
- 923 Tan EK, Yew K, Chua E, Puvan K, Shen H, Lee E, et al. PINK1 mutations in sporadic early-
924 onset Parkinson's Disease. *Mov. Disord.* 2006; 21: 789–793.
- 925 Taymans J-M, Greggio E. LRRK2 Kinase Inhibition as a Therapeutic Strategy for Parkinson's
926 Disease, Where Do We Stand? *Curr. Neuropharmacol.* 2016; 14: 214–225.
- 927 Trinh J, Zeldenrust FMJ, Huang J, Kasten M, Schaake S, Petkovic S, et al. Genotype-phenotype
928 relations for the Parkinson's disease genes SNCA, LRRK2, VPS35: MDSGene systematic
929 review. *Mov. Disord.* 2018
- 930 Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, Frontali M, et al. Localization of
931 a Novel Locus for Autosomal Recessive Early-Onset Parkinsonism, PARK6, on Human
932 Chromosome 1p35-p36. *Am. J. Hum. Genet.* 2001; 68: 895–900.
- 933 Valente EM, Salvi S, Ialongo T, Marongiu R, Elia AE, Caputo V, et al. PINK1 mutations are
934 associated with sporadic early-onset Parkinsonism. *Ann. Neurol.* 2004; 56: 336–341.
- 935 Vilas D, Sharp M, Gelpi E, Genís D, Marder KS, Cortes E, et al. Clinical and neuropathological
936 features of progressive supranuclear palsy in Leucine rich repeat kinase (*LRRK2*) G2019S
937 mutation carriers. *Mov. Disord.* 2017; 00: 1–3.
- 938 Wickremaratchi MM, Perera D, O'Loughlen C, Sastry D, Morgan E, Jones a, et al. Prevalence
939 and age of onset of Parkinson's disease in Cardiff: a community based cross sectional study and
940 meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 2009a; 80: 805–807.
- 941 Wickremaratchi MM, Perera D, O'Loughlen C, Sastry D, Morgan E, Jones A, et al. Prevalence
942 and age of onset of Parkinson's disease in Cardiff: a community based cross sectional study and
943 meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 2009b; 80: 805–807.
- 944 Williams-Gray CH, Goris A, Foltynie T, Brown J, Maranian M, Walton A, et al. Prevalence of
945 the LRRK2 G2019S mutation in a UK community based idiopathic Parkinson's disease cohort.
946 *J. Neurol. Neurosurg. Psychiatry* 2006; 77: 665–667.
- 947 Wszolek ZK, Pfeiffer B, Fulgham JR, Parisi JE, Thompson BM, Uitti RJ, et al. Western
948 Nebraska family (family D) with autosomal dominant parkinsonism. *Neurology* 1995; 45: 502–
949 505.
- 950 Zabetian CP, Hutter CM, Yearout D, Lopez AN, Factor SA, Griffith A, et al. LRRK2 G2019S in
951 families with Parkinson disease who originated from Europe and the Middle East: evidence of
952 two distinct founding events beginning two millennia ago. *Am. J. Hum. Genet.* 2006a; 79: 752–

- 953 8.
- 954 Zabetian CP, Morino H, Ujike H, Yamamoto M, Oda M, Maruyama H, et al. Identification and
955 haplotype analysis of LRRK2 G2019S in Japanese patients with Parkinson disease. *Neurology*
956 2006b; 67: 697–699.
- 957 Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, et al. A clinic-based study
958 of the LRRK2 gene in Parkinson disease yields new mutations. *Neurology* 2005; 65: 741–744.
- 959 Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2
960 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 2004; 44: 601–
961 607.
- 962